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<p>(54) Title: TRIGEMINAL SENSORY STIMULI AND ANIMAL REPELLENTS FROM PLANTS</p> <p>(57) Abstract</p> <p>Novel uses for compounds isolated from the fruit of <i>Xanthoxylum</i> and <i>echinacea</i> species, and similar compounds from other spice and flowering species, and the oil extracts from which they are isolated, are disclosed. The novel uses include flavor enhancers, additives for oral, hair, and skin care products, and animal repellents.</p>		

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TRIGEMINAL SENSORY STIMULI AND ANIMAL REPELLENTS FROM PLANTS

This is a continuation-in-part of application Serial No. 08/792,276, filed January 31, 1997, incorporated by reference herein in its entirety.

5 Field of the Invention

The present invention relates to sensory irritants and repellents in solvent extracts from certain plant species.

Background of the Invention

- 10 Extracts of the fruits of *Xanthoxylum* (also spelled "Zanthoxylum" in the literature) and *Echinacea* species of plants have been used to repel and deter insects, in anti-fungal applications, and to prepare medicine for toothaches and stomachs. Bowers et al., *J. of Nat. Prod.*, 56(6): 935-938, June, 1993 report the isolation and identification of insect repellent/deterrent components from the essential oil of the fruit *Xanthoxylum*
- 15 *hungeanum*, commonly known as Chinese prickly ash. The fruit itself is a peppery spice used in Chinese cooking. Although novel monoterpene compounds were reported as identified-- 4-terpinenyl acetate, α -terpinenyl acetate, and caryophyllene -- it was found that the previously identified monoterpenes contributed the most to insect repellency. The compounds exhibited insect repellent activity against ants of the genus *Crematogaster*.
- 20 No isolation or description of alkylamides was described.

The isolation and characterization of sanshoamide from *Xanthoxylum piperitum* is described by Aihara, in *Yakugaku Zasshi*, 71:1112, 1951. The paper reports slight pungency of the alcohol solution of the compound, and does not report any pungency from the crystalline compound. Sanshoamide was obtained from the non-

- 2 -

volatile fraction of the ethereal extract of fresh, unripe, fruit collected in early summer. The structure of sanshoamide is represented as N-2'-hydroxyl-2,4,8, 10-dodecatetraene-amide-1'.

2, 6, 8, 10- dodecatetraenoic acid is an insecticidal compound isolated from *Zanthoxylum* species and *Echinacea angustifolia* roots. The all-E form of this compound is commonly known as β -Sanshool, also an insect repellent. Dictionary of Natural Products, Vol. 2, D-F, p. 1939. Chapman & Hall, London, 1994. Also reported therein is 2, 4, 8, 10 -dodecatetraenoic acid from which sanshoamide is derived. Chapman & Hall, *supra*.

Dube et al., *Annals of Botany*, 65:457 - 459, 1990 describe the antifungal and insect-repellent activity of the essential oil of *Zanthoxylum alatum*. The insect-repellent activity of the essential oil was tested against *Allacophora foveicollis* Fabr.

Tachiyashiki et al., *J. of Japanese Society of Nutrition and Food Science*, 45(2):123-128, April, 1992 describe the effects of the scent and/or appearance of *kinome* (Japanese pepper leaf, i.e., *Zanthoxylum piperitum*) and lemon peel on human whole saliva secretion.

Bohlmann et al., *Phytochemistry*, 22(5): 1173-1175, 1983, report upon the isolation of novel amides from *Echinacea purpurea*. The previous isolation of highly unsaturated amides is referenced. The novel amides reported are as follows:

1. trideca-2t, 7c-dien-10, 12 - diynoic acid isobutylamide;
2. pentadeca-2t, 9c-dien-12, 14-diynoic acid isobutylamide;
3. trideca -2t, 7c-dien-10,12-diynoic acid (2-methylbutyl)amide;
4. pentadeca-2t, 9c-dien-12, 14-diynoic acid (2-hydroxy isobutyl)-amide;
- and
5. trideca-2t,6t, 8c-trien-10,12-diynoic acid isobutylamide.

There is no discussion of the biological effect/function of these compounds.

Yasuda et al., *Chem. Pharm. Bull.*, 29(2):564-566, 1981, report on the isolation of three unsaturated aliphatic acid amides from the roots of *Asiasarum heterotropoides* Mack. var *mandshuricum* Mack. The amides isolated are the following:

1. (2E, 4E) - N-isobutyl-2,4 -decadienamide (pellitorine);
2. (2E, 4E, 8Z, 10E) - N- isobutyl-2,4,8,10- dodecatetraenamide, and

3. (2E, 4E, 8Z, 10Z)- N-isobutyl -2,4,8,10- dodecatetraenamide.

There is no discussion of their biological effect.

Yasuda et al., *Phytochemistry*, 21(6):1295-1298, 1982 describes an amide isolated from several *Zanthoxylum* species named hydroxy- α -sanshool which is 2-
5 hydroxy-N-isobutyl-dodeca 2E, 6Z, 8E, 10E-tetraenamide. This amide, and others, was described as a strong pungent principle.

Pfander et al., *Deutsche Apotheker Zeitung*, 2381-2384, 1987, describes, *inter alia*, pungent compounds isolated from Szechuan pepper, a type of *Zanthoxylum*, belonging to the class alkylamides. The authors report that the samples they had were
10 completely free of pungent compounds when tested organoleptically, and that a comparison with fresh fruits from *Z. alatum*, proved that the acid amides tasted extraordinarily sharp and can lead to a clear anesthesia of the mucosa even in small amounts.

Fenaroli's Handbook of Flavor Ingredients, 2nd Edition, Thomas E. Furia
15 and Nicolo Bellanca, eds., CRC Press, p. 445, 1975 reports that prickly ash bark extract -- i.e., from species *Xanthoxylum americanum* Mill and *X. clava-herculis* L. -- is a bitter tonic and aromatic which actually twinges the tongue when taken into the mouth. Its use in cordials, non-alcoholic beverages, candy, and baked-goods is described.

JP 58-213706 describes oral compositions for the prevention of dental
20 plaque containing, *inter alia*, sanshool. The compositions are reported to inhibit formation of deposits or detritus caused by bacteria. Amounts of 0.001-5 weight percent are indicated. Use in toothpaste, tooth powders, liquid dentrifices, mouthwashes, coating agents, and chewing gums are described

JP 01-294657 describes an anesthetic agent isolated from *Zanthoxylum*
25 *bungeanum* which showed anesthetic activity when 610 mg were applied to the tongue. The anesthetic compound is reported as having the formula 2'-hydroxy-N-isobutyl 2,4,5,11-tetradecatetraenamide. The reference also describes the occurrence of hydroxy- α -sanshool in the extract.

JP 06-298659 describes topical preparations containing sanshool and
30 sanshamide from *Zanthoxylum piperitum* to enhance sexual activity. The preferred mixing ratio of extract from the Japanese pepper is indicated as 20-50 weight percent

A crystalline compound isolated from *Echinacea angustifolia*, called echinacein, which produced excessive salivation and an intense, burning, paralytic effect on the tongue and on the mucous membranes of the lips and mouth in trace amounts has been described. *J. Org. Chem.*, 32(5):1646-7, 1967. The structure was reported to be
5 identical to α -sanshool and neoherculin.

JP 07-090294 describes the use of essential oils rich in spilanthol for manufacturing toothpastes or other oral compositions. The structure for spilanthol is described as (2E,6Z,8E)-N-isobutyl-2,6,8-decatrienamide. Yasuda et al. *Chem. Pharm. Bull.*, 28(7)2251-2253, 1980.

10 JP 6211675 and 6-211676 describe the use of sanshool extract from *Zanthoxylum piperitum* in a composition for treating impotence. The composition preferably contains 5-90% of sanshool extract.

U.S. Patent No. 4,639,368, issued to Niazi et al. on January 27, 1987, describes the addition of up to 5 mg/individual portion of spilanthol to chewing gum
15 adapted to supply a medicament. The spilanthol is reported to serve as an anaesthetic to help mask the taste of medicaments having particularly strong, unpleasant tastes.

Summary of the Invention

We have discovered, and disclose herein, that extracts from the genera *Xanthoxylum* and *Echinacea* have certain stimulatory effects in humans and, further,
20 deterrent effects in other animals. We have identified the active fraction of these extracts as comprising alkylamides. We have isolated three compounds from an extract of *Xanthoxylum* and, thus far, have structurally characterized one compound -- 2,6,8,10-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide. The structure of this compound is the same as that identified as hydroxy- α -sanshool discussed above.

25 In one aspect, the present invention relates to an additive for products for human consumption comprising alkylamide-containing extracts isolated from certain species of plants.

In another aspect, the present invention relates to an additive for products for human consumption comprising alkylamides isolatable from certain species of plants.

30 In another aspect, the present invention relates to an additive for oral-, hair-

and skin-care products comprising an alkylamide-containing extract isolated from certain species of plants.

In another aspect, the present invention relates to an additive for oral-, hair- and skin-care products comprising alkylamides isolatable from certain species of
5 plants.

In yet another aspect, the present invention relates to a method for enhancing the sensory effect of oral-, hair- or skin-care products comprising the addition of alkylamide-containing extracts isolated from certain species of plants.

In yet another aspect, the present invention relates to a method for
10 enhancing the sensory effect of products for human consumption comprising the addition of alkylamide-containing extracts isolated from certain species of plants.

In a further aspect, the present invention relates to a method for enhancing the sensory effect of oral-, hair- or skin-care products comprising the addition of alkylamides isolatable from certain species of plants.

In a further aspect, the present invention relates to a method for
15 enhancing the sensory effect of products for human consumption comprising the addition of alkylamides isolatable from certain species of plants.

In yet a further aspect, the present invention relates to a method for repelling non-human animals comprising the application of alkylamide-containing
20 extracts isolated from certain species of plants to materials susceptible to damage by these animals.

In yet a further aspect, the present invention relates to a method for repelling non-human animals comprising the application of alkylamides isolatable from certain species of plants to materials susceptible to rodent damage.

In another aspect, the present invention relates to a method for stimulating
25 certain thermal and/or mechanosensory neurons in the skin or mouth of an animal comprising the application of alkylamide-containing extracts from certain species of plants and, therefore, altering or inducing thermal or tactile sensation.

In another aspect, the present invention relates to a method for stimulating
30 certain thermal and/or mechanosensory neurons in the skin or mouth of an animal

comprising the application of alkylamides isolatable from certain species of plants and, therefore, altering or inducing thermal or tactile sensation.

Brief Description of the Drawings

Figure 1- Multiwavelength chromatogram of a semipurified extract of *Xanthoxylum* obtained from Nepal. Two of the peaks of the complex of 3 peaks at approximately 10 minutes are active in human sensory tests.

Figure 2- Responses of cultured trigeminal sensory neurons to 15 seconds of stimulation with pentanoic acid (pH 7.0), pH 6.0 HEPES, zingerone (10 μ M), capsaicin (100 nM), hydroxy- α -sanshool (4.8 μ g/ml), and KCl (50 mM). Note that neurons are differentially sensitive to capsaicin (Neuron 3) and hydroxy- α -sanshool (Neurons 1 and 2). However, one neuron (Neuron 1) is sensitive to both protons and hydroxy- α -sanshool.

Figures 3A-B Activation of sensory neurons by hydroxy- α -sanshool. Action potentials were recorded from the lingual branch of the trigeminal nerve of rat. The frequency of action potentials increases from panel A to B. The neuron (action potentials) indicated above was also sensitive to moderate cooling.

Figures 4A-B Effect of hydroxy- α -sanshool on tactile responses of trigeminal neurons. Light tactile stimuli are indicated by arrows. **4A.** Action potentials before application of hydroxy- α -sanshool to the tongue. **4B.** Action potentials approximately 4 minutes after application of hydroxy- α -sanshool to the tongue. The frequency of action potentials increased during each brief tactile stimulation.

Figures 5A-B Activation of a cold nociceptor (indicated by arrow) by hydroxy- α -sanshool, causing it to fire bursts of 2-4 action potentials at normal tongue temperature.

35°C. Panel A is before treatment of tongue with hydroxy- α -sanshool, panel B is 4 minutes after treatment.

Detailed Description

We found that novel sensations are elicited in the mouth by solvent
5 extracts from species of *Xanthoxylum*, including, but not limited to, *X. alatum* and *X. americanum*, and *Echinacea*, including, but not limited to, *E. angustifolia* and *E. purpurea*, in humans, and have further identified the responsible compounds therein -- alkylamides. It is expected that other plant species contain alkylamides similar to these, i.e. similar in effect. The extracts can be prepared from the fruit, roots, leaves, and
10 bark of the plants. In a preferred embodiment, the solvent used is ethyl acetate, although other solvents can be used. Notably, after evaporation of the solvent, the extracts elicited sensations that are unlike the irritation produced by the commonly used sensory irritants -- i.e., capsaicin, piperine, and zingerone.

Xanthoxylum and *Echinacea* extracts were tested by drying equal
15 volumes of solvent extracts on filter paper and testing on several human subjects for the intensity and character of sensation. Separation of the substances in these extracts, using HPLC, yielded several active peaks that are active when sampled on the tongue or in the mouth when dissolved in ethanol. The sensations produced by the active substances are different than those produced by ethanol alone. While ethanol is characterized by
20 sensations of heat and pain, substances in *Xanthoxylum* and *Echinacea* elicited sensations varyingly described as numbness, tingling, enhanced cooling on inspiration of air, a 'buzzing' type of irritation, and, in some subjects, pain.

We also tested these extracts for repellent effect in non-human animals. In two rodent species tested (Plains pocket gopher and rat), the subjects rejected
25 desirable food (e.g., pieces of apple) that had been coated with an oil extract of *Xanthoxylum* fruits. The rats, after contacting the food, displayed behaviors characteristic of oral rejection -- i.e., gaping and oral grooming behavior. Pocket gophers rejected the food before oral contact and vigorously buried the food in their

cage.

Black-tailed deer (*Odocoileus hemionus columbianus*) were repelled from eating apples treated with extract. Their initial repulsion occurred without oral contact of the food/extract, suggesting that volatile compounds contained in the extract are
5 repellent in this species.

The extracts were also found to be repellent in birds.

Given the sensory nature of these extracts, we disclose their use, and the use of the active compounds therein, as human sensory stimuli, for, for example, increasing the sensory impact and/or adding "freshness" to oral-, hair-, or skin-care
10 products, and products for human consumption. Contemplated uses for oral-, hair-, and skin-care products include, but are not limited to, flavor modifiers or enhancers for use in dentrifices, dental floss, mouthwashes; additives for, for example, shampoos, preferably dandruff shampoos; and topical analgesic creams, and massage oils. Contemplated uses for human consumption include, but are not limited to, breath mints,
15 throat lozenges, confections (e.g. chewing gum, "fire" ball candies), ice creams, oral medications -- e.g., those which require chewing or sucking -- etc.

The extracts and compounds are included in these products to produce increased sensory impact and/or induce a novel feeling of freshness similar in effect, but distinctly different, from the pungency of pepper or the cooling of mint. The tingling
20 feeling disclosed herein produced by the extracts/compounds is distinct from an anesthetic effect (i.e., the lessening of a feeling) and is a desirable sensation for many oral-care products and some food items. For such uses, the extract of non-volatile compounds is preferably used to eliminate potentially odorous or bitter compounds that may have an undesirable impact.

25 Because similarly pungent extracts/compounds have been demonstrated in plant species in addition to *Xanthoxylum* and *Echinacea*, extracts/compounds from these additional species having the similar effect are also included within the scope of the invention. Extracts containing both volatile and/or non-volatile compounds are suitable for such uses.

30 An amount of the extract or compound sufficient to enhance the sensory effect of the product to which it is added is to be used. Such an amount will stimulate

thermal and/or mechanosensory neurons, without necessarily stimulating high threshold pain receptors, in the mouth or on the skin or scalp. This amount will be readily ascertainable by persons of skill in the art. An exemplary amount for the extract is about 5 to about 50 microliters/gram of sample; an exemplary concentration range of the alkylamide is about 150 to about 500 micrograms/100ml H₂O.

Moreover, because a strong aversive reaction by rodents, deer, and birds was observed to these extracts, their use, and the use of the compounds isolatable therefrom, as animal repellents is disclosed. For such use, the volatile and non-volatile extracts are suitable. The extracts and compounds can be used to reduce or eliminate depredation of materials generally susceptible to depredation including, but not limited to, crops, livestock feedlot, crops, seeds, seedlings, telephone cables, electrical cables, containers for discarded refuse, packaging, fabrics, and plastics.

Essentially, "depredation", as used herein, refers to contact an animal may have with materials which causes some destruction or diminution in value of said materials, including consumption.

An agent is repellent if it substantially reduces depredation of a material as compared to depredation of the same material in the absence of such repellent agent. As will be understood by those skilled in the art, the value of a repellent is ultimately determined by the value of the material under depredation. For some valuable materials, a reduction of about 25% in the rate of predation is considered substantial. Those of ordinary skill in the art will recognize methods of testing to determine the amount of compound which will provide the desired repellency effect.

The term "extract" as used herein refers to the material collected by extraction of plant matter using appropriate solvent(s).

The term "isolated" as used herein means separated from materials with which the compound is normally associated in the native state.

The phrase "isolatable by HPLC" as used herein refers to a compound having the same, or substantially the same, structure as a compound obtained in the manner specified. Accordingly, synthetically produced compounds having the same, or substantially the same, structure are also contemplated and within the scope of the invention. By "substantially the same structure" is meant compounds having minor

- 10 -

changes in structure, but retaining the effect described herein.

The terms "topical" or "topically" as used herein refer to external application.

Example 1

5 Isolation of the active compounds from Xanthoxylum fruit

An ethyl acetate extract was obtained by extracting 60 grams of dried fruits 3x with 800 ml ethyl acetate, and then evaporating this material using a rotary evaporator (bath temperature 40°C) to give an oil-like, black-brown liquid with a characteristic aromatic odor, i.e., the "ethyl acetate extract." 10.7 grams of this liquid
10 was obtained. This extract was substituted into ethanol and suspended in oil for testing of repellent properties against birds, deer, and voles.

The liquid extract was applied to a column (30x400 mm) packed with silica gel (Davisil grade 634 (Fisher), 100-200 mesh) and chromatographed in 5% EtOH in hexane. The collected fractions were analyzed by High Performance Thin Layer
15 Chromatography ("HPTLC"). The fractions having activity when applied to the human tongue ($R_f = 0.5$ in 20% EtOH in hexane, plates: Kieselgel GF254 (Merck); UV(254nm) - adsorbed spot) were combined and evaporated to give a brown-colored, oil-like liquid with a weak odor (1.0 g). This material was re-chromatographed, using the same conditions as described above, to give a brown liquid (0.9 g) with almost no
20 odor, i.e., the "alkylamide extract." This material was tested for repellent properties in rats after suspension in EtOH (5 ml) and vegetable oil (50 ml). Rat chow (Wayne Rodent Blocks) was soaked in the oil suspension until the food was saturated. The food absorbed 7.6% of its weight of oil suspension.

The alkylamide extract was further separated into individual compounds
25 for structure determination and bioassay. The alkylamide extract (0.9 g, above) was diluted in 90% EtOH (2 ml) and filtered through a C18 cartridge (preparative size, Fisher). The filtrate was chromatographed (2x 1ml) using reverse-phase HPLC. The conditions of HPLC separation were as follows: 1) Column: Zorbax C18 (21.2 x 250 mm), 2) mobile phase: 35% iPrOH in water, 3) flow: 10 ml/min, and 4) detection: high
30 speed scanning 200-360 nm. The fractions containing a major peak were combined.

- 11 -

evaporated down to 80% of original volume, diluted with water (3:1) and individual compounds were isolated by reverse-phase extraction on C18 cartridge. Adsorbed substances were eluted with MeOH (5 ml). The methanolic solution was analyzed by HPTLC using the conditions described above. HPTLC showed a single spot (both in
5 UV and by detection using H_2SO_4). Analysis by HPLC revealed a complex of three peaks in the methanolic solution (Figure 1). The compound from the major (central) peak, i.e., compound 1, was isolated using preparative reverse-phase HPLC and its structure is described below.

The stability of the isolated compound, as measured by High Pressure
10 Liquid Chromatography ("HPLC"), could be significantly improved by the presence of stabilizers such as 4-methyl-2,6-di-*t*-butyl-phenol, during extraction and final storage.

Example 2

Structure determination of the isolated compound

The Mass Spectroscopy ("MS") spectrum obtained by chemical ionization
15 gave a peak 264, which is ascribable as MH^+ , and MS spectrum with ionization by electron impact (EI) gave a peak 263. The 1H - ^{13}C heteronuclear COSY Nuclear Magnetic Resonance ("NMR") and ^{13}C -DEPT NMR showed the presence of 3 methyls, 3 methylenes, 8 methynes and 2 quaternary carbon atoms.

The Infrared Spectroscopy ("IR") spectra showed signals at 1675 cm^{-1} ,
20 (amide I), 1550 cm^{-1} (amide II), and the presence of an amide group was also suggested by carbon chemical shift. From further IR data --in film; broad singlet 3300 cm^{-1} -- and MS data -- chemical ionization: 246 m/z : $MH-18$; EI: 245 m/z $M-18$ -- we concluded that an -OH group is present. From the foregoing data, we established the molecular formula as $C_{16}H_{25}NO_2$.

25 The NMR data of the compound are disclosed in the Table I. Chemical shifts are reported in units of ppm and coupling constants in hertz.

- 12 -

TABLE I

Assignment	¹³ C chemical shift	Proton chemical shift in CD ₃ OD
1	169.2 -CO(NH or O)	
2	145.2 =CH	6.8 dt 16, 7.5 Hz
3	134.9 =CH	6.15 m
4	133.4 =CH	6.15 m
5	125.3 =CH	6.0 d(t-long range) 16, (1.5) Hz
6	130.77 =CH	5.7 dq 15, 7.5 Hz
7	130.62 =CH	5.4 dt 11.5, 7.5 Hz
8	126.7 =CH	6.36 dd 12.5, 11.5 Hz
9	131.1 =CH	6.0 dd 12.5 11.5 Hz
10	71.8 C-O(N)	
11	51.23 CH ₂ -N(O)	3.2 s
12	33.3 CH ₂ -	2.3dt 7.5 Hz
13	27.7 CH ₂ -	2.35dt 7.5 Hz
14,15	27.4 2 (-CH ₃)	1.2 s
16	18.6 -CH ₃	1.75 d 7.5Hz

From ¹H-¹H COSY NMR and several homonuclear decoupling NMR spectra the following fragments were established:

20 Fragment 1:

C(16)-C(6)-C(3 or 4)-C(4 or 3)-C(8)-C(9)-C(7)-C(13)-C(12)-C(2)-C(5)-.

that is:

Me-CH=CH-CH=CH-CH=CH-CH₂-CH₂-CH=CH-

E E Z E

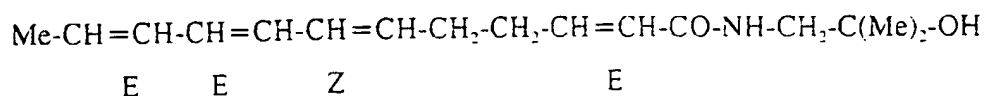
25 Fragment 2:

C(14)-C(10)-C(15),
that is : (N or O)-C(Me)₂-.

C,H-COLOC NMR revealed long range coupling ($J=7$ Hz) between C1 and C11. Thus, the amide moiety of the molecule was concluded to be:

5 C1-N-C11-C10-(C14, C15).

From these data, we concluded that compound 1 has the following structure:



or

10 N-(2-methyl-2-hydroxypropyl)-dodeca-(2E, 6Z, 8E, 10E)-tetraenamide, or 2,6,8,10-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide. This compound has been previously described as hydroxy- α -sanshool.

Example 3

15 Biological activity of hydroxy- α -sanshool

The activity of hydroxy- α -sanshool was determined by its ability to induce changes in intracellular calcium in characterized trigeminal sensory neurons, *in vitro*. The measurement of intracellular calcium was performed as described in U.S. Patent Application No. 08/541,641, incorporated herein by reference. Hydroxy- α -
20 sanshool induced increases in calcium in neurons (high threshold pain receptors) that were sensitive to capsaicin, the pungent principle of the hot pepper and neurons that were insensitive to capsaicin. (Figure 2).

We have also determined the neural activity using neurophysiological recordings from the lingual branch of the trigeminal nerve of rat. This nerve branch
25 mediates thermal, mechanical, and pain sensation on the tongue. The procedure utilized is described in Komai et al., *Brain Research*, 612: 122-129, 1993, hereby incorporated by reference. Hydroxy- α -sanshool increased the spontaneous activity in thermal and mechanosensory neurons (Figures 3 and 4) which are sensitive to moderate cooling or light tactile stimulation, respectively. In this preparation, high threshold pain receptors

- 14 -

were not always stimulated. This finding is significant because stimulation of these classes of neurons without the activation of higher threshold pain receptors may contribute to the unique sensory character of these extracts/compounds. Hydroxy- α -sanshool also increased the responses of tactile neurons to light touch (Figure 4).

- 5 Finally, hydroxy- α -sanshool activated a high threshold cold receptor (cold nociceptor) to fire bursts of action potentials (Figure 5), an activity that is characteristic of cold-sensitive neurons that have been activated by very low (e.g., $< 10-15^{\circ}\text{C}$) temperature stimuli. This finding is significant because activation of these low threshold sensory neurons, without activation of other classes of high threshold nociceptors, may also
10 contribute to the unique sensations of these extracts.

Example 4

Repellent effect on rodents

- Over the course of a 4 day feeding experiment (two choice test, each spice extract vs. EtOH/oil control treated food), 20 hr food-deprived rats (6 rats/spice)
15 consumed significantly less rat chow that had been treated with a crude vegetable oil suspension of *Xanthoxylum* extract than rat chow that had been treated with a similar oil suspension of an extract made of the equal weight of cinnamon. Cinnamon extract was included as a control for novelty. *Xanthoxylum* extract suppressed feeding by 98.7% (amount of treated food/total food consumed by each rat per day) while cinnamon extract
20 suppressed feeding by 82.5%. Thus, while extracts of both *Xanthoxylum* and cinnamon reduce food intake, the *Xanthoxylum* extract was more aversive.

Example 5

Repellent effect on birds

- Treated and untreated (control) dog food was presented to a wild
25 population of magpies, *Pica pica* for 8 hours per day for 5 days. Treated dog food was prepared using the ethyl acetate extract as described above. Significantly less treated food was consumed than control food (3-way, repeated measures, ANOVA, $p < .01$). This response indicates that the extract is repellent to magpies and, it is expected, repellent to birds in general.

The foregoing examples are meant to illustrate the invention, not limit it. Those skilled in the art will recognize modifications which are within the spirit and scope of the inventions as set forth in the appended claims.

5 All references cited herein are hereby incorporated by reference in their entirety.

What is claimed is:

1. An additive for increasing the sensory impact and/or inducing a feeling of freshness in products for human consumption, said additive comprising an effective amount of an alkylamide-containing extract from species of plants selected from the group consisting
5 of *Echinacea*, and other species of plants containing similar alkylamides.
2. An additive for increasing the sensory impact and/or inducing a feeling of freshness in products for human consumption, said additive comprising an effective amount of at least one alkylamide isolatable by HPLC from solvent extracts from species of plants selected from the group consisting of *Xanthoxylum*, *Echinacea*, and other species of
10 plants containing similar alkylamides.
3. The additive of claim 2 wherein said additive comprises 2E,6Z,8E,10E-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide.
4. An additive for increasing the sensory impact and/or inducing a feeling of freshness in oral-, hair-, or skin-care products, said additive comprising an effective amount of an
15 extract from species of plants selected from the group consisting of *Xanthoxylum* and *Echinacea*.
5. An additive for increasing the sensory impact and/or inducing a feeling of freshness in oral-, hair-, or skin-care products, said additive comprising an effective amount of at least one alkylamide isolatable by HPLC from solvent extracts from species of plants
20 selected from the group consisting of *Xanthoxylum* and *Echinacea*.
6. The additive for oral-, hair-, or skin-care products of claim 5 wherein said additive comprises 2E,6Z,8E,10E-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide.
7. A method for enhancing the sensory effect of oral-, hair-, or skin-care products comprising adding an effective amount of an alkylamide-containing extract from species

of plants selected from the group consisting of *Xanthoxylum* and *Echinacea*.

8. A method for enhancing the sensory effect of oral-, hair-, or skin-care products comprising adding an effective amount of at least one alkylamide isolatable by HPLC from solvent extracts from species of plants selected from the group consisting of
5 *Xanthoxylum* and *Echinacea*.
9. The method of claim 7 or 8 wherein said extract or alkylamide is added in an amount sufficient to stimulate one or more thermal and/or mechanosensory receptors.
10. A method for enhancing the sensory effect of products for human consumption comprising adding an effective amount of an alkylamide-containing extract from species
10 of plants selected from the group consisting of *Xanthoxylum*, *Echinacea*, and other species of plants containing similar alkylamides.
11. A method for enhancing the sensory effect of products for human consumption comprising adding an effective amount of at least one alkylamide isolatable by HPLC from solvent extracts from species of plants selected from the group consisting of
15 *Xanthoxylum*, *Echinacea*, and other species of plants containing similar alkylamides.
12. The method of claim 10 or 11 wherein said extract or alkylamide is added in an amount sufficient to stimulate one or more thermal and/or mechanosensory receptors.
13. The method of claim 8 or 11 wherein said at least one alkylamide is 2E.6Z.8E.10E-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide.
- 20 14. A method for repelling non-human animals from a material susceptible to their depredation comprising administering an effective amount of an alkylamide-containing extract from species of plants selected from the group consisting of *Xanthoxylum*.

- 18 -

Echinacea, and other plants containing similar alkylamides, to said material.

15. The method of claim 14 wherein said extract is an ethyl acetate extract as described herein.

5 16. The method of claim 14 wherein said extract is an alkylamide extract as described herein.

17. A method for repelling non-human animals from a material susceptible to their depredation comprising administering an effective amount of an alkylamide isolatable by HPLC from solvent extracts from species of plants selected from the group consisting of
10 *Xanthoxylum*, *Echinacea*, and other plants containing similar alkylamides, to said material.

18. The method of claim 17 wherein said at least one alkylamide is 2E,6Z,8E,10E-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide.

19. A method for stimulating one or more thermal and/or mechanosensory receptors
15 selected from the group consisting of moderate cooling, high threshold cold, and light tactile receptors in the skin or mouth of an animal comprising providing an effective amount of at least one alkylamide isolatable by HPLC from solvent extracts from species of plants selected from the group consisting of *Xanthoxylum*, *Echinacea*, and other plants containing similar alkylamides, to said animal orally or topically.

20 20. A method for stimulating one or more thermal and/or mechanosensory receptors other than high threshold pain receptors in the skin or mouth of an animal comprising providing an effective amount of at least one alkylamide isolatable by HPLC from solvent extracts from species of plants selected from the group consisting of
25 *Xanthoxylum*, *Echinacea*, and other plants containing similar alkylamides, to said animal orally or topically.

- 19 -

21. The method of claim 19 or 20 wherein said at least one alkylamide is 2E,6Z,8E,10E-dodecatetraenoic acid, N-2-hydroxy-2-methylpropylamide.

1/5

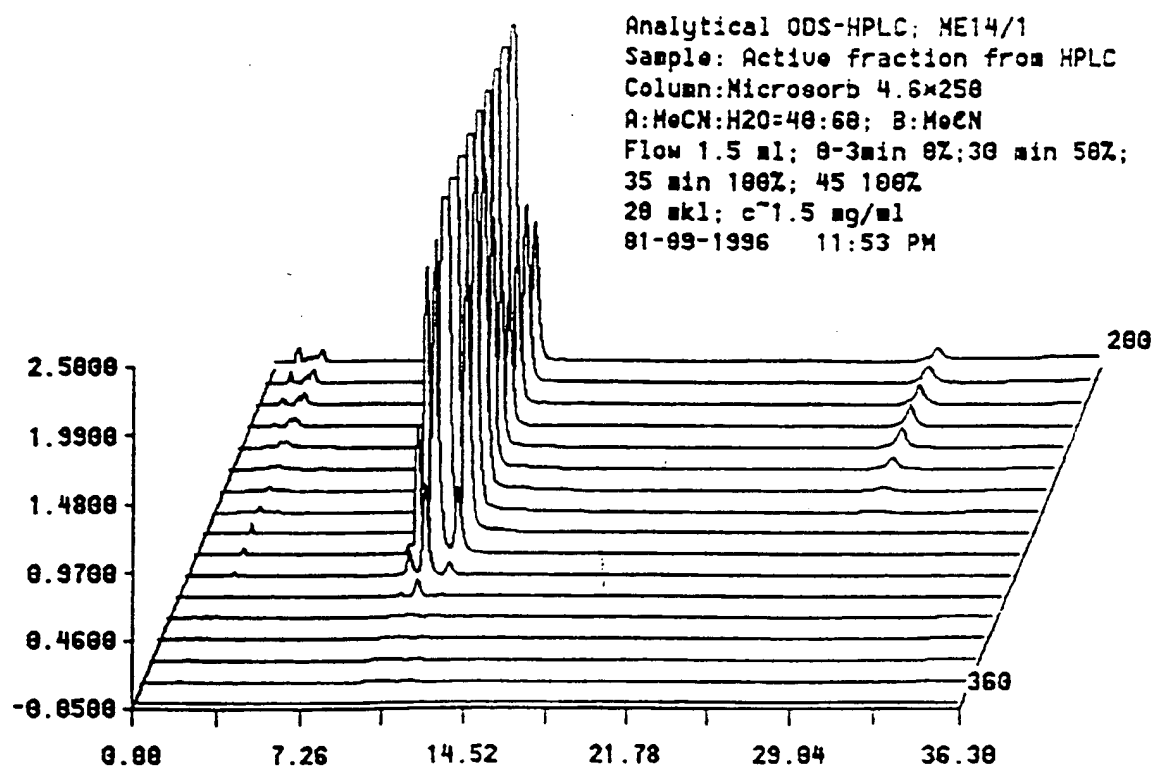


FIGURE 1

2/5

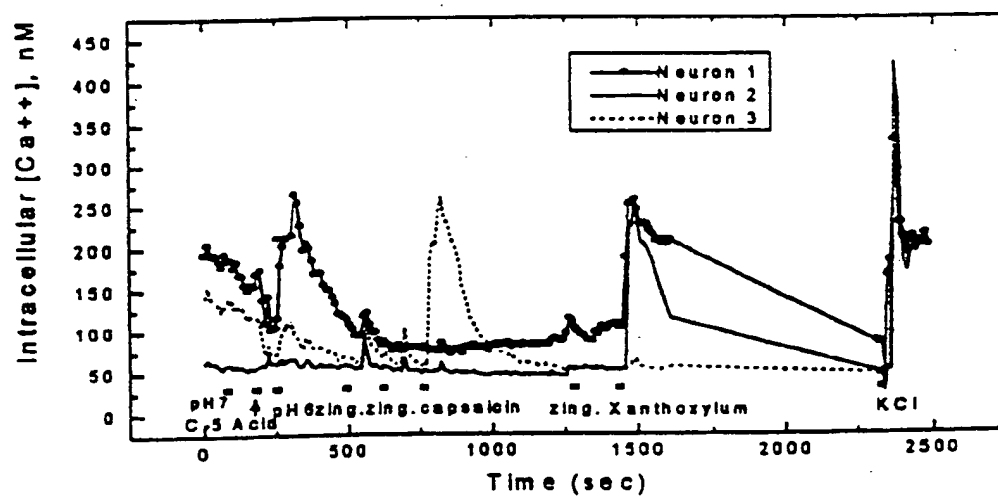


FIGURE 2

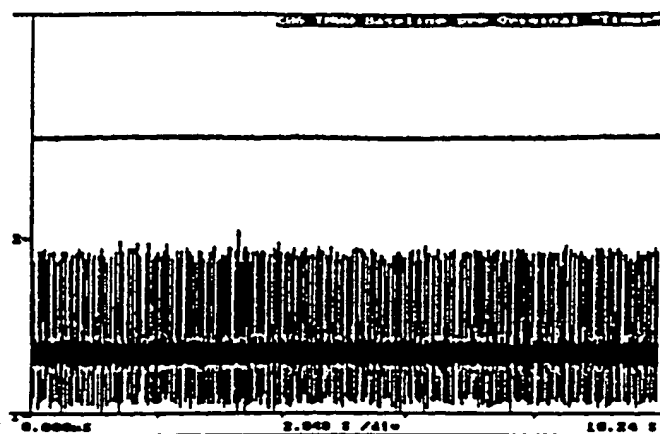


FIGURE 3A

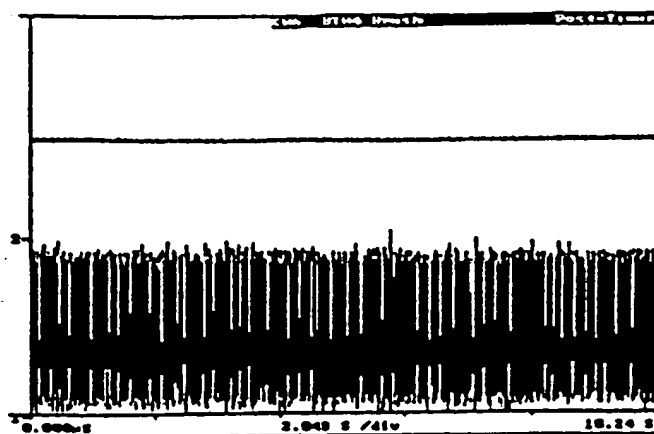


FIGURE 3B

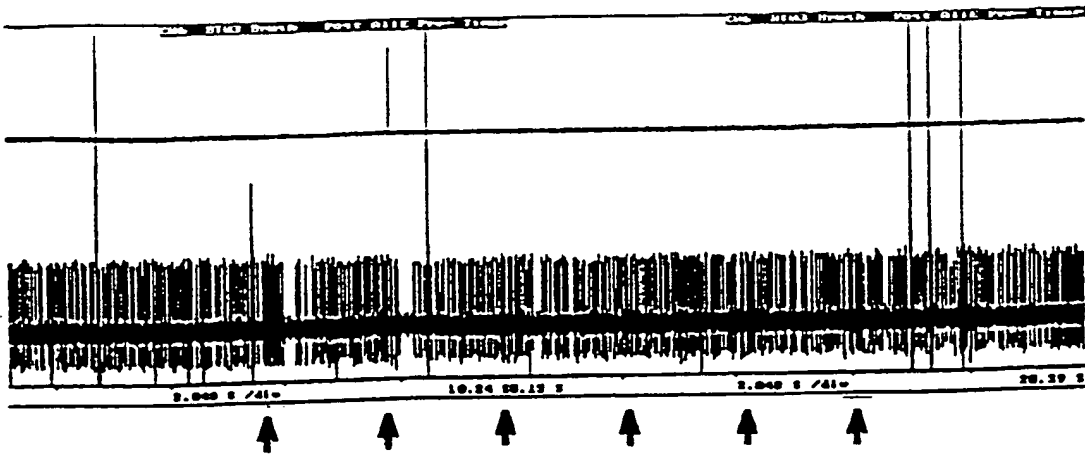


FIGURE 4A

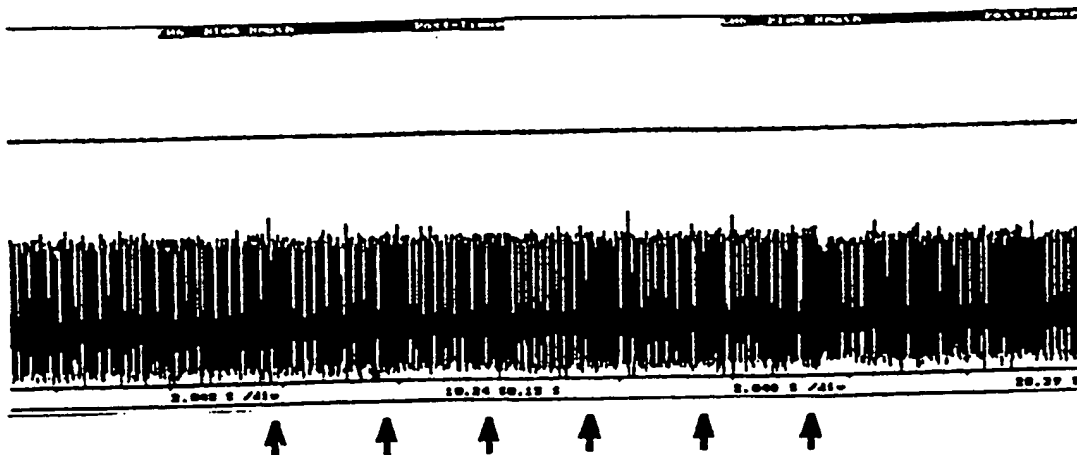


FIGURE 4B

5/5

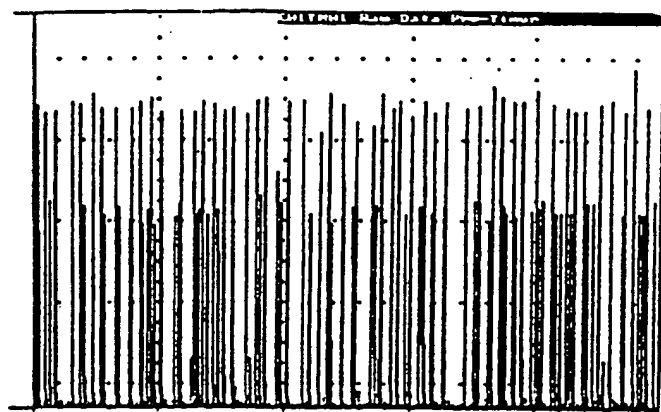


FIGURE 5A

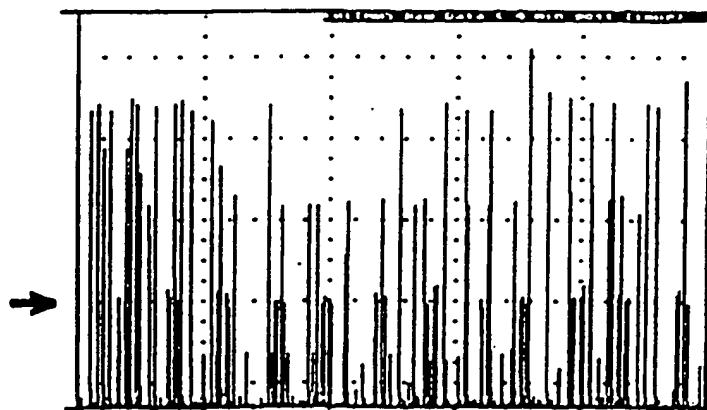


FIGURE 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22537

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 65/00

US CL :424/195.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/195.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,401,502 A (WUNDERLICH et al.) 28 MARCH 1995, see Example 3.	1-21



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 JANUARY 1999

Date of mailing of the international search report

29 JAN 1999

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22537

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS on line

CAS on line

WPIDS on line

USPATFULL on line